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(54) **ENVIRONMENTAL STRESS TOLERANCE GENES**

TOLERANZGENE FÜR UMWELTSTRESS

GENES RESISTANT AU STRESS ENVIRONNEMENTAL

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(73) Proprietor: **Mendel Biotechnology, Inc.**
Hayward, CA 94545-3720 (US)

(72) Inventors:

- **PINEDA, Omaira**
Vero Beach, FL 32960 (US)
- **YU, Guo-Liang**
Berkeley, CA 94705 (US)
- **CREELMAN, Robert**
Castro Valley, CA 94546 (US)
- **RIECHMANN, Jose, Luis**
Pasadena, CA 91101 (US)
- **HEARD, Jacqueline**
Stonington, CT 06378-1722 (US)
- **RATCLIFFE, Oliver**
Oakland, CA 94606 (US)
- **REUBER, Lynne**
San Mateo, CA 94402 (US)
- **KEDDIE, James**
San Mateo, CA 94402 (US)

(74) Representative: **Brasnett, Adrian Hugh et al**
Mewburn Ellis LLP
York House
23 Kingsway
London WC2B 6HP (GB)

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description**FIELD OF THE INVENTION**

[0001] This invention relates to the field of plant biology. More particularly, the present invention pertains to compositions and methods for phenotypically modifying a plant.

BACKGROUND OF THE INVENTION

[0002] Transcription factors can modulate gene expression, either increasing or decreasing (inducing or repressing) the rate of transcription. This modulation results in differential levels of gene expression at various developmental stages, in different tissues and cell types, and in response to different exogenous (e.g., environmental) and endogenous stimuli throughout the life cycle of the organism.

[0003] Kasuga et al., 1999, *Nature Biotechnology*, Vol. 17, pp. 287-291, describes gene transfer of a single stress-inducible transcription factor.

[0004] Because transcription factors are key controlling elements of biological pathways, altering the expression levels of one or more transcription factors can change entire biological pathways in an organism. For example, manipulation of the levels of selected transcription factors may result in increased expression of economically useful proteins or metabolic chemicals in plants or to improve other agriculturally relevant characteristics. Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a plant's traits.

[0005] Described herein are novel transcription factors useful for modifying a plant's phenotype in desirable ways, such as modifying a plant's environmental stress tolerance.

SUMMARY OF THE INVENTION

[0006] In one aspect, the invention is a transgenic plant as defined in the claims.

[0007] The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

[0008] Furthermore, the invention relates to a method for producing a plant having improved environmental stress tolerance, the method being as defined in the claims.

BRIEF DESCRIPTION OF THE DRAWINGS**[0009]**

Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences. Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences. Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

DETAILED DESCRIPTION

[0010] The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

[0011] In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance, such as freezing, chilling, heat, drought, water saturation, salt, photocon-

ditions, radiation and ozone, or the like. Plants with altered expression of the polynucleotides or polypeptides of the invention are more tolerant to these environmental stresses compared with plants without altered expression levels.

[0012] The polynucleotides disclosed herein encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4: 1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 1996 250:7-16), the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-I protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

[0013] In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides described herein, the polynucleotides and polypeptides have a variety of additional uses. These uses include their use in the recombinant production (i.e., expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

DEFINITIONS

[0014] A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA, a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

[0015] A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

[0016] An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

[0017] A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

[0018] The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeroplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

[0019] A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises

a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant,

as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell. **[0020]** The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides:

[0021] The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

[0022] The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

[0023] "Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

[0024] Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, molluscs, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenolipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time, flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

POLYPEPTIDES AND POLYNUCLEOTIDES

[0025] Described herein are among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's environmental stress tolerance.

[0026] Exemplary polynucleotides encoding the polypeptides were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

[0027] Additional polynucleotides were identified by screening *Arabidopsis thaliana* and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

[0028] The polynucleotides were ectopically expressed in overexpressor or knockout plants and changes in the environmental stress tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the environmental stress resistance of plants.

Making polynucleotides

[0029] The polynucleotides include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or inteins, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

[0030] A variety of methods exist for producing the polynucleotides. Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

[0031] Alternatively, polynucleotides can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis). Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, *all supra*.

[0032] Alternatively, polynucleotides and oligonucleotides can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides

are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

5 HOMOLOGOUS SEQUENCES

[0033] Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from *Arabidopsis thaliana* or from other plants of choice are also described herein. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such pine, poplar and eucalyptus.

[0034] Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

Identifying Nucleic Acids by Hybridization

[0035] Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

[0036] An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

[0037] As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will

depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

[0038] Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

SEQUENCE VARIATIONS

[0039] It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides described herein. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

[0040] For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

Table 1

Amino acids			Codon						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	TGC	TGT					
Aspartic acid	Asp	D	GAC	GAT					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	TTC	TTT					
Glycine	Gly	G	GGA	GGC	GGG	GGT			
Histidine	His	H	CAC	CAT					
Isoleucine	Ile	I	ATA	ATC	ATT				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT	
Methionine	Met	M	ATG						
Asparagine	Asn	N	AAC	AAT					
Proline	Pro	P	CCA	CCC	CCG	CCT			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT	
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT	
Threonine	Thr	T	ACA	ACC	ACG	ACT			
Valine	Val	V	GTA	GTC	GTG	GTT			
Tryptophan	Trp	W	TGG						
Tyrosine	Tyr	Y	TAC	TAT					

[0041] Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art.

[0042] In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide.

[0043] For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) *Meth. Enzymol.* (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substi-

tutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

[0044] Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

Table 2

Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser; Val
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

[0045] Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

FURTHER MODIFYING SEQUENCES MUTATION/FORCED EVOLUTION

[0046] In addition to generating silent or conservative substitutions as noted, above, described herein are methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

[0047] Thus, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) Nature 370:389-391, and Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Many other mutation and evolution methods are also available and

expected to be within the skill of the practitioner.

[0048] Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

[0049] Accordingly, the specification provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

[0050] For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

[0051] The polynucleotide sequences can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

[0052] Furthermore, a fragment or domain derived from any of the polypeptides described herein can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor described herein can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7: 1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51: 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

EXPRESSION AND MODIFICATION OF POLYPEPTIDES

[0053] Typically, polynucleotide sequences are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

Vectors, Promoters and Expression Systems

[0054] Described herein are recombinant constructs comprising one or more of the nucleic acid sequences disclosed herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

[0055] General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642, for dicotyledonous plants.

[0056] Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced.

An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) *Plant Physiol* 102: 1077-1084; Vasil (1993) *Bio/Technology* 10: 667-674; Wan and Lemeaux (1994) *Plant Physiol* 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) *Nature Biotech* 14: 745-750).

[0057] Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

[0058] Examples of constitutive plant promoters which can be useful for expressing the TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (see, e.g., Odel et al. (1985) *Nature* 313:810); the nopaline synthase promoter (An et al. (1988) *Plant Physiol* 88:547); and the octopine synthase promoter (Fromm et al. (1989) *Plant Cell* 1: 977).

[0059] A variety of plant gene promoters that regulate gene expression in response to environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known promoters have been characterized and can favorably be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the *dru 1* promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No. 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) *Plant Mol Biol* 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) *Plant Mol Biol* 37:977-988), flower-specific (Kaiser et al. (1995) *Plant Mol Biol* 28:231-243), pollen (Baerson et al. (1994) *Plant Mol Biol* 26:1947-1959), carpels (Ohl et al. (1990) *Plant Cell* 2:837-848), pollen and ovules (Baerson et al. (1993) *Plant Mol Biol* 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) *Plant Mol Biol* 39:979-990 or Baumann et al. (1999) *Plant Cell* 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) *Plant Mol Biol* 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) *Plant Mol Biol* 38:1053-1060, Willmott et al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) *Plant Mol Biol* 22: 13-23), light (e.g., the pea *rbcS-3A* promoter, Kuhlemeier et al. (1989) *Plant Cell* 1:471, and the maize *rbcS* promoter, Schaffner and Sheen (1991) *Plant Cell* 3: 997); wounding (e.g., *wun1*, Siebertz et al. (1989) *Plant Cell* 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) *Plant Mol. Biol.* 40:387-396, and the PDF1.2 promoter described in Manners et al. (1998) *Plant Mol. Biol.* 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) *Plant Mol Biol* 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) *Science* 270: 1986-1988); or late seed development (Odell et al. (1994) *Plant Physiol* 106:447-458).

[0060] Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

Additional Expression Elements

[0061] Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

Expression Hosts

[0062] The present invention also relates to plant cells which are transduced with vectors described herein and the production of polypeptides (including fragments thereof) by recombinant techniques. Host cells are genetically engineered

(i.e., nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors disclosed herein, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

[0063] The host cell can be a plant cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of *Agrobacterium tumefaciens* or *A. rhizogenes* carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

[0064] The cell can include a nucleic acid which encodes a polypeptide, wherein the cells expresses a polypeptide described herein. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

[0065] For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins described herein can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

Modified Amino Acids

[0066] Polypeptides may contain one or more modified amino acids. The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

[0067] Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

IDENTIFICATION OF ADDITIONAL FACTORS

[0068] A transcription factor can also be used to identify additional endogenous or exogenous molecules that can affect a phenotype or trait of interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue is expressed in a host cell, e.g., a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al. (1999) Nature Biotechnology 17:573-577).

[0069] The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

[0070] The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

IDENTIFICATION OF MODULATORS

[0071] In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northern, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

[0072] Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

[0073] In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

[0074] A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) Nature Biotechnology, 14(3): 309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. Science (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum C&EN Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

[0075] Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, Int. J. Pept. Prot. Res. 37:487-493 (1991) and Houghton et al. Nature 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

[0076] In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries

useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

[0077] Indeed, entire high throughput screening systems are commercially available. These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

[0078] The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators that have an effect on one or more polynucleotides or polypeptides according to the present invention.

[0079] In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

[0080] Described herein is a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell, plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

SUBSEQUENCES

[0081] Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like, according to methods as noted *supra*.

[0082] Subsequences of the polynucleotides described herein including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides, or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

[0083] In addition, described herein are isolated or recombinant polypeptides including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides disclosed herein. For example, such polypeptides, or domains or fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

PRODUCTION OF TRANSGENIC PLANTS

Modification of Traits

[0084] The polynucleotides disclosed herein are favorably employed to produce transgenic plants with various traits,

or characteristics, that have been modified in a desirable manner, e.g., to improve the environmental stress resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) described herein, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved environmental stress tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

Antisense and Cosuppression Approaches

[0085] In addition to expression of the nucleic acids disclosed herein as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) *Antisense Technology: A Practical Approach* IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

[0086] For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably, the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

[0087] Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No. 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

[0088] Vectors in which RNA encoded by a transcription factor or transcription factor homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

[0089] Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to suppress expression of an endogenous transcription factor, thereby reducing or eliminating its activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) *Genes and Development* 13: 139-141).

[0090] Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) *Methods in Arabidopsis Research*, World Scientific).

[0091] Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription

factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

[0092] A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised.

If the lox sites are in the opposite orientation, the intervening sequence is inverted.

[0093] The polynucleotides and polypeptides described herein can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

[0094] The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

[0095] Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides and/or expressing the polypeptides described herein can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

[0096] The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean, clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae* (melon and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture -Crop Species. Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

[0097] Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

[0098] Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

[0099] Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

[0100] After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide described herein can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

INTEGRATED SYSTEMS-SEQUENCE IDENTITY

[0101] Also described herein is an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such improved environmental stress tolerance, with one or more identified

sequence.

[0102] For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot

and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched. **[0103]** Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., *supra*.

[0104] A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

[0105] One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length *W* in the query sequence, which either match or satisfy some positive-valued threshold score *T* when aligned with a word of the same length in a database sequence. *T* is referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters *M* (reward score for a pair of matching residues; always > 0) and *N* (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity *X* from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters *W*, *T*, and *X* determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (*W*) of 11, an expectation (*E*) of 10, a cutoff of 100, *M*=5, *N*=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (*W*) of 3, an expectation (*E*) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

[0106] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (*P(N)*), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

[0107] The integrated system, or computer typically includes a user input interface allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

[0108] The methods can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

[0109] Thus, described herein are methods for identifying a sequence similar or homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a

sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

[0110] Any sequence herein can be entered into the database, before or after querying the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

EXAMPLES

[0111] The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

[0112] Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

[0113] Alternatively, *Arabidopsis thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60° C) and labeled with ³²p dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO₄ pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60°C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSSC, 1% SDS at 60° C.

[0114] To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon™ cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon™ Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

[0115] Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

[0116] The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with Sall and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

[0117] Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini Prep kits (Qiagen, CA).

EXAMPLE III. TRANSFORMATION OF AGROBACTERIUM WITH THE EXPRESSION VECTOR

[0118] After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of *Agrobacterium tumefaciens* cells for transformation were

made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance (A_{600}) of 0.5 - 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4°C. Cells were then resuspended in 250 μ l chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were centrifuged again as described above and resuspended in 125 μ l chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 μ l and 750 μ l, respectively. Resuspended cells were then distributed into 40 μ l aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

[0119] *Agrobacterium* cells were transformed with plasmids prepared as described above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 - 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 μ l of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 μ F and 200 μ F using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 - 4 hours at 28°C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 μ g/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28°C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

EXAMPLE IV. TRANSFORMATION OF ARABIDOPSIS PLANTS WITH AGROBACTERIUM TUMEFACIENS WITH EXPRESSION VECTOR

[0120] After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance (A_{600}) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044 μ M benzylamino purine (Sigma), 200 μ l/L Silwet L-77 (Lehle Seeds) until an absorbance (A_{600}) of 0.8 was reached.

[0121] Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~ 10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75 μ E/m²/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

[0122] The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

[0123] Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H₂O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H₂O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75 μ E/m²/sec) at 22-23° C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T₁ generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

[0124] Primary transformants were crossed and progeny seeds (T₂) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

[0125] The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 base pairs to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

EXAMPLE VII. IDENTIFICATION OF ENVIRONMENTAL STRESS TOLERANCE PHENOTYPE IN OVEREX-PRESSOR OR GENE KNOCKOUT PLANTS

[0126] Experiments were performed to identify those transformants or knockouts that exhibited an improved environmental stress tolerance. For such studies, the transformants were exposed to a variety of environmental stresses. Plants were exposed to chilling stress (6 hour exposure to 4-8°C), heat stress (6 hour exposure to 32-37°C), high salt stress (6 hour exposure to 200 mM NaCl), drought stress (168 hours after removing water from trays), osmotic stress (6 hour exposure to 3 M mannitol), or nutrient limitation (nitrogen, phosphate, and potassium) (Nitrogen: all components of MS medium remained constant except N was reduced to 20mg/L of NH₄ NO₃, or Phosphate: All components of MS medium except KH₂ PO₄, which was replaced by K₂SO₄, Potassium: All components of MS medium except removal of KNO₃ and KH₂PO₄, which were replaced by NaH₄PO₄).

[0127] Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

Table 3

SEQ ID No.	GID	Knockout (KO) or overexpressor (OX)	Phenotype observed
1	G22	OE	Increased tolerance to high salt
3	G188	KO	Better germination under osmotic stress
5	G225	OE	Increased tolerance to nitrogen-limited medium
7	G226	OE	Increased tolerance to nitrogen-limited medium
9	G256	OE	Better germination and growth in cold
11	G419	OE	Increased tolerance to potassium-free medium
13	G464	OE	Better germination and growth in heat
15	G482	OE	Increased tolerance to high salt
17	G502	KO	Increased sensitivity to osmotic stress
19	G526	OE	Increased sensitivity to osmotic stress
21	G545	OE	Susceptible to high salt
23	G561	OE	Increased tolerance to potassium-free medium
25	G664	OE	Better germination and growth in cold
27	G682	OE	Better germination and growth in heat
29	G911	OE	Increased growth on potassium-free medium
31	G964	OE	Better germination and growth in heat
33	G394	OE	More sensitive to chilling
35	G489	OE	Increased tolerance to osmotic stress

[0128] For a particular overexpressor that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with an increased expression of the particular transcription factor.

EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

[0129] Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) J. Mol. Biol. 215:403-410; and Altschul et al. (1997) Nucl. Acid Res. 25: 3389-3402). The tblastx sequence analysis programs were employed using the BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) Proc. Natl. Acad. Sci. USA 89:0915-10919).

[0130] Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of 3.6e-40 is 3.6×10^{-40} . For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

[0131] In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-*Arabidopsis* genes shown in Figure 3 and the *Arabidopsis* genes in the sequence listing are: SEQ ID No. 1: 53%-67%; SEQ ID No. 3: 38%-76%; SEQ ID No. 5: 34%-67%; SEQ ID No. 7: 50%-69%; SEQ ID No. 9: 32%-91%; SEQ ID No. 11: 48%-66%; SEQ ID No. 13: 34%-60%; SEQ ID No. 15: 58%-81%; SEQ ID No. 17: 65%-94%; SEQ ID No. 19: 72%-83%; SEQ ID No. 21: 52%-64%; SEQ ID No. 23: 40%-89%; SEQ ID No. 25: 86%-97%; SEQ ID No. 27: 41%-75%; SEQ ID No. 29: 29%-72%; SEQ ID No. 31: 49%-70%; SEQ ID No. 33: 56%-86%; SEQ ID No. 35: 61%-84%; SEQ ID No. 37: 40%-58%; SEQ ID No. 39: 63%-87%; SEQ ID No. 41: 51%-88%; SEQ ID No. 43: 80%-90%; SEQ ID No. 45: 79%-90%; SEQ ID No. 47: 30%-58%; SEQ ID No. 49: 52%-62%; SEQ ID No. 51: 55%-73% and SEQ ID No. 53: 44%-80%.

[0132] The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the environmental stress tolerance of a plant.

SEQUENCE LISTING

[0133]

<110> Pineda, Omaira
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Riechmann, Jose Luis
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Ratcliffe, Oliver
Reuber, Lynne
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Arg Arg Arg Asp Phe Phe Arg Lys
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Leu Ile Ser Arg Met Tyr Arg Leu Val Gly Asn Arg Trp Asp Leu Ile
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Ala Gly Arg Val Val Gly Arg Lys Ala Asn Glu Ile Glu Arg Tyr Trp
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80 85 90

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Gln Asn Pro Thr Asp His His His Tyr Asn His Gln Ile Phe Gly Ser
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 Asp Gln Leu Ile Arg Val Glu Pro Glu Ser Leu Ser Ser Ile Val Thr
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Gly Ser Lys Arg Ser Ala Glu Ser Ser Ser His Gln Gly Ala Ser Pro
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Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Leu His Arg
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Glu Gly Asp Gly Glu Lys Lys Val Val Lys Asn Gly Glu Leu Lys Asp
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 Leu Gln Arg Phe Arg Glu Ile Glu Gly Glu Arg Thr Gly Leu Gly Arg
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 Pro Gln Thr Gly Gly Glu Val Gly Glu His Gln Arg Asp Ala Val Gly
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 Asp Gly Gly Gly Phe Tyr Gly Gly Gly Gly Gly Met Gln Tyr His Gln
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      Glu Phe Ile Ser Phe Val Thr Gly Glu Ala Ser Asp Lys Cys Gln Lys
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	Leu Gln Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu	
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	Pro Gly Leu Ala Leu Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro	
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	agg gac aga aaa tat ccc aac ggt tcg cgt cct aac cgg tcc gct ggt	475
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	acg gag tca gga tac tgg aaa gcc acc ggg aag gat aag gag atc ttc Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe	516
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	115 120 125	
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	130 135 140	
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	145 150 155 160	
35	agg gac gaa tgg gtc gtg tgt agg gtt ttt cac aag aac aat cct tct Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Asn Pro Ser	708
	165 170 175	
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	180 185 190	
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	195 200 205	
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	210 215 220	
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	225 230 235 240	
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	245 250 255	
	ggg tct gct tcg ggc tct acg tac aac aac aac aac gag atg atc aag Gly Ser Ala Ser Gly Ser Thr Tyr Asn Asn Asn Asn Glu Met Ile Lys	996
	260 265 270	
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	Asp Val Asn Ala Asn Met Thr Thr Thr Glu Val Ser Ser Gly Pro	
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	gta atg aaa caa gaa atg ggg atg atg gga atg gtg aat ggt agc aag	1140
	Val Met Lys Gln Glu Met Gly Met Met Gly Met Val Asn Gly Ser Lys	
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	tcg tat gaa gat cta tgt gac ttg agg ggg gac ttg tgg gac ttc taa	1188
	Ser Tyr Glu Asp Leu Cys Asp Leu Arg Gly Asp Leu Trp Asp Phe	
	325 330 335	
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	tattatttaa ttagttgatt gtttaattag ttatacttt atagtgtggt taaaaagaa	1368
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5 Glu Leu Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu
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Glu Ile Ile Thr Cys Tyr Leu Lys Glu Lys Val Leu Asn Ser Arg Phe
35 40 45

10 Thr Ala Val Ala Met Gly Glu Ala Asp Leu Asn Lys Cys Glu Pro Trp
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Asp Leu Pro Lys Arg Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe
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15 Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala
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20 Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe
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Lys Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr
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Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Asn Pro Ser

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	165	170	175
5	Thr Thr Thr Gln Pro Met Thr Arg Ile Pro Val Glu Asp Phe Thr Arg 180 185 190		
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	Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe 245 250 255		
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	Met Glu Gln Ser Leu Val Ser Val Ser Gln Glu Thr Cys Leu Ser Ser 275 280 285		
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		20 25 30
10	ggc aag cga tct aag aga tca aga tcc gat ttc cac cac caa aac ctc Gly Lys Arg Ser Lys Arg Arg Ser Asp Phe His His Gln Asn Leu	201
		35 40 45
15	act gag gaa gag tat cta gct ttt tgc ctc atg ctt ctc gct cgc gac Thr Glu Glu Glu Tyr Leu Ala Phe Cys Leu Met Leu Leu Ala Arg Asp	249
		50 55 60 65
20	aac cgt cag cct cct cct cct ccg gcg gtg gag aag ttg agc tac aag Asn Arg Gln Pro Pro Pro Pro Pro Ala Val Glu Lys Leu Ser Tyr Lys	297
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	tgt agc gtc tgc gac aag acg ttc tct tct tac caa gct ctc ggt ggt Cys Ser Val Cys Asp Lys Thr Phe Ser Ser Tyr Gln Ala Leu Gly Gly	345
		85 90 95
25	cac aag gca agc cac cgt aag aac tta tca cag act ctc tcc ggc gga His Lys Ala Ser His Arg Lys Asn Leu Ser Gln Thr Leu Ser Gly Gly	393
		100 105 110
30	gga gat gat cat tca acc tcg tcg gcg aca acc aca tcc gcc gtg act Gly Asp Asp His Ser Thr Ser Ser Ala Thr Thr Ser Ala Val Thr	441
		115 120 125
	act gga agt ggg aaa tca cac gtt tgc acc atc tgt aac aag tct ttt Thr Gly Ser Gly Lys Ser His Val Cys Thr Ile Cys Asn Lys Ser Phe	489
		130 135 140 145
35	cct tcc ggt caa gct ctc gcc gga cac aag ccg tgc cac tac gaa gga Pro Ser Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu Gly	537
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	aac aac aac atc aac act agt agc gtg tcc aac tcc gaa ggt gcg ggg Asn Asn Asn Ile Asn Thr Ser Ser Val Ser Asn Ser Glu Gly Ala Gly	585
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40	tcc act agc cac gtt agc agt agc cac cgt ggg ttt gac ctc aac atc Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn Ile	633
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		195 200 205
	agc cct atg ccg gcg aag aag cct ccg ttt gac ttt ccg gtc aaa ctt Ser Pro Met Pro Ala Lys Lys Pro Arg Phe Asp Phe Pro Val Lys Leu	729
		210 215 220 225
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	Gln Leu	
	ttagagattgt ttaggaatttt gttgactgta cataccaaat tggactttga ctgattccaa	838
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Lys Gly Lys Arg Ser Lys Arg Ser Arg Ser Asp Phe His His Gln Asn
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Leu Thr Glu Glu Glu Tyr Leu Ala Phe Cys Leu Met Leu Leu Ala Arg
50 55 60

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Asp Asn Arg Gln Pro Pro Pro Pro Pro Ala Val Glu Lys Leu Ser Tyr
65 70 75 80

Lys Cys Ser Val Cys Asp Lys Thr Phe Ser Ser Tyr Gln Ala Leu Gly
85 90 95

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Gly His Lys Ala Ser His Arg Lys Asn Leu Ser Gln Thr Leu Ser Gly
100 105 110

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Gly Gly Asp Asp His Ser Thr Ser Ser Ala Thr Thr Thr Ser Ala Val
115 120 125

Thr Thr Gly Ser Gly Lys Ser His Val Cys Thr Ile Cys Asn Lys Ser
130 135 140

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Phe Pro Ser Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu
145 150 155 160

Gly Asn Asn Asn Ile Asn Thr Ser Ser Val Ser Asn Ser Glu Gly Ala
165 170 175

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Gly Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn
180 185 190

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Ile Pro Pro Ile Pro Glu Phe Ser Met Val Asn Gly Asp Asp Glu Val
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Leu Gln Leu
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Met Gly Ser Asn Glu Glu Gly Asn Pro
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act aac aac tct gat aag cca tcg caa gct gct gct cct gag cag agt 160
Thr Asn Asn Ser Asp Lys Pro Ser Gln Ala Ala Ala Pro Glu Gln Ser
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aat gtt cat gtg tat cat cat gac tgg gct gct atg cag gca tat tat 208
Asn Val His Val Tyr His His Asp Trp Ala Ala Met Gln Ala Tyr Tyr
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ggg cct aga gtt ggt ata cct caa tat tac aac tca aat ttg gcg cct 256
Gly Pro Arg Val Gly Ile Pro Gln Tyr Tyr Asn Ser Asn Leu Ala Pro
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ggt cat gct cca ccg cct tat atg tgg gcg tct cca tcg cca atg atg 304
Gly His Ala Pro Pro Pro Tyr Met Trp Ala Ser Pro Ser Pro Met Met
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[illegible]

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5 Ser Gln Ala Ala Ala Pro Glu Gln Ser Asn Val His Val Tyr His His
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Asp Trp Ala Ala Met Gln Ala Tyr Tyr Gly Pro Arg Val Gly Ile Pro
35 40 45

10 Gln Tyr Tyr Asn Ser Asn Leu Ala Pro Gly His Ala Pro Pro Pro Tyr
50 55 60

Met Trp Ala Ser Pro Ser Pro Met Met Ala Pro Tyr Gly Ala Pro Tyr
65 70 75 80

15 Pro Pro Phe Cys Pro Pro Gly Gly Val Tyr Ala His Pro Gly Val Gln
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20 Met Gly Ser Gln Pro Gln Gly Pro Val Ser Gln Ser Ala Ser Gly Val
100 105 110

Thr Thr Pro Leu Thr Ile Asp Ala Pro Ala Asn Ser Ala Gly Asn Ser
115 120 125

25 Asp His Gly Phe Met Lys Lys Leu Lys Glu Phe Asp Gly Leu Ala Met
130 135 140

Ser Ile Ser Asn Asn Lys Val Gly Ser Ala Glu His Ser Ser Ser Glu
145 150 155 160

30 His Arg Ser Ser Gln Ser Ser Glu Asn Asp Gly Ser Ser Asn Gly Ser
165 170 175

35 Asp Gly Asn Thr Thr Gly Gly Glu Gln Ser Arg Arg Lys Arg Arg Gln
180 185 190

Gln Arg Ser Pro Ser Thr Gly Glu Arg Pro Ser Ser Gln Asn Ser Leu
195 200 205

40 Pro Leu Arg Gly Glu Asn Glu Lys Pro Asp Val Thr Met Gly Thr Pro
210 215 220

45 Val Met Pro Thr Ala Met Ser Phe Gln Asn Ser Ala Gly Met Asn Gly
225 230 235 240

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Val Pro Gln Pro Trp Asn Glu Lys Glu Val Lys Arg Glu Lys Arg Lys
245 250 255

5 Gln Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Leu Arg Lys Gln Ala
260 265 270

Glu Thr Glu Gln Leu Ser Val Lys Val Asp Ala Leu Val Ala Glu Asn
275 280 285

10 Met Ser Leu Arg Ser Lys Leu Gly Gln Leu Asn Asn Glu Ser Glu Lys
290 295 300

15 Leu Arg Leu Glu Asn Glu Ala Ile Leu Asp Gln Leu Lys Ala Gln Ala
305 310 315 320

Thr Gly Lys Thr Glu Asn Leu Ile Ser Arg Val Asp Lys Asn Asn Ser
325 330 335

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	ccg tgc tgt gag aaa gct cac aca aac aaa gga gca tgg acg aaa gaa	163
	Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala Trp Thr Lys Glu	
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	tgg aga tct ctc ccc aaa gcc gcc gga ctt ctt cgc tgt ggc aag agc	259
	Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser	
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15	tgc cgt ctc cgg tgg atc aac tat ctc cgg cct gac ctt aag cgt gga	307
	Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly	
	55 60 65	
	aac ttc acc gag gaa gaa gac gaa ctc atc atc aag ctc cat agc ctt	355
	Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu	
20	70 75 80	
	ctt ggc aac aaa tgg tgc ctt att gcc ggg aga tta ccg gga aga aca	403
	Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu Pro Gly Arg Thr	
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25	gat aac gag ata aag aac tat tgg aac acg cat ata cga aga aag ctt	451

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	Ile	Asn	Arg	Gly	Ile	Asp	Pro	Thr	Ser	His	Arg	Pro	Ile	Gln	Glu	Ser	
				120					125					130			
	tca	gct	tct	caa	gat	tct	aaa	cct	aca	caa	cta	gaa	cca	gtt	acg	agt	547
	Ser	Ala	Ser	Gln	Asp	Ser	Lys	Pro	Thr	Gln	Leu	Glu	Pro	Val	Thr	Ser	
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	Asn	Thr	Ile	Asn	Ile	Ser	Phe	Thr	Ser	Ala	Pro	Lys	Val	Glu	Thr	Phe	
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	cat	gaa	agt	ata	agc	ttt	ccg	gga	aaa	tca	gag	aaa	atc	tca	atg	ctt	643
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	Thr	Phe	Lys	Glu	Glu	Lys	Asp	Glu	Cys	Pro	Val	Gln	Glu	Lys	Phe	Pro	
					185					190					195		
20	gat	ttg	aat	ctt	gag	ctc	aga	atc	agt	ctt	cct	gat	gat	gtt	gat	cgt	739
	Asp	Leu	Asn	Leu	Glu	Leu	Arg	Ile	Ser	Leu	Pro	Asp	Asp	Val	Asp	Arg	
				200					205					210			
	ctt	caa	ggg	cat	gga	aag	tca	aca	acg	cca	cgt	tgt	ttc	aag	tgc	agc	787
25	Leu	Gln	Gly	His	Gly	Lys	Ser	Thr	Thr	Pro	Arg	Cys	Phe	Lys	Cys	Ser	
				215			220						225				
	tta	ggg	atg	ata	aac	ggc	atg	gag	tgc	aga	tgc	gga	aga	atg	aga	tgc	835
	Leu	Gly	Met	Ile	Asn	Gly	Met	Glu	Cys	Arg	Cys	Gly	Arg	Met	Arg	Cys	
				230			235					240					
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	Asp	Phe	Leu	Gly	Leu	Ala	Lys	Lys	Glu	Thr	Thr	Ser	Leu	Leu	Gly	Phe	
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35	cga	agc	ttg	gag	atg	aaa	taa	tattgtcaaa	ttttaggcgt	aactgtacaa							982
	Arg	Ser	Leu	Glu	Met	Lys											
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	aacttttgcc	tagataattt	gaaagtatat	cttcaacttg	tatgagaaat	tttaactggtg											1042
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Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
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50 55 60

15 Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys
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20 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu
85 90 95

Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile
100 105 110

25 Arg Arg Lys Leu Ile Asn Arg Gly Ile Asp Pro Thr Ser His Arg Pro
115 120 125

Ile Gln Glu Ser Ser Ala Ser Gln Asp Ser Lys Pro Thr Gln Leu Glu
130 135 140

30 Pro Val Thr Ser Asn Thr Ile Asn Ile Ser Phe Thr Ser Ala Pro Lys
145 150 155 160

35 Val Glu Thr Phe His Glu Ser Ile Ser Phe Pro Gly Lys Ser Glu Lys
165 170 175

Ile Ser Met Leu Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gln
180 185 190

40 Glu Lys Phe Pro Asp Leu Asn Leu Glu Leu Arg Ile Ser Leu Pro Asp
195 200 205

45 Asp Val Asp Arg Leu Gln Gly His Gly Lys Ser Thr Thr Pro Arg Cys
210 215 220

Phe Lys Cys Ser Leu Gly Met Ile Asn Gly Met Glu Cys Arg Cys Gly
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Ser Asn Gly Phe Asp Phe Leu Gly Leu Ala Lys Lys Glu Thr Thr Ser
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act tct tct tct gaa gaa gtg agt agt ctt gag tgg gaa gtt gtg aac	96
Thr Ser Ser Ser Glu Glu Val Ser Ser Leu Glu Trp Glu Val Val Asn	
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atg agt caa gaa gaa gaa gat ttg gtc tct cga atg cat aag ctt gtc	144
Met Ser Gln Glu Glu Glu Asp Leu Val Ser Arg Met His Lys Leu Val	
35 40 45	

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Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala	
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gga gaa att gag agg ttt tgg gtc atg aaa aat tga	228
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Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala
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Gly Glu Ile Glu Arg Phe Trp Val Met Lys Asn
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15	ata tta aag ata ctt tac gtc atc ggt ttc ttt aga gac atg gtc gat	96
	Ile Leu Lys Ile Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp	
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	Ala Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu	
	35 40 45	
25	acc tct gga ccc gat ccg acc cga cac gct ctc tct acg tca gcg agt	192
	Thr Ser Gly Pro Asp Pro Thr Arg His Ala Leu Ser Thr Ser Ala Ser	
	50 55 60	
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	Leu Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Pro Thr	
	65 70 75 80	
35	gat ccg gaa gat tgt tgt acg gtt tgt ttg tca gat ttt gag tcc gac	288
	Asp Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Glu Ser Asp	
	85 90 95	
40	gat aag gtt agg cag cta ccc aag tgt gga cac gtg ttt cat cat cat	336
	Asp Lys Val Arg Gln Leu Pro Lys Cys Gly His Val Phe His His His	
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Tyr Gly Gln Met Thr Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys
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	Asp	Met	Phe	Phe	Arg	Thr	Asn	Pro	Gly	Thr	Val	Gly	Leu	Thr	Ser	Gln	165	170	175	
35	Phe	Thr	Lys	Pro	Leu	Arg	Leu	Leu	Asp	Gly	Ser	Ser	Glu	Phe	Val	Leu	180	185	190	
40	Thr	Tyr	Glu	Asp	Lys	Glu	Gly	Asp	Trp	Met	Leu	Val	Gly	Asp	Val	Pro	195	200	205	
	Trp	Arg	Met	Phe	Ile	Asn	Ser	Val	Lys	Arg	Leu	Arg	Val	Met	Lys	Thr	210	215	220	
45	Ser	Glu	Ala	Asn	Gly	Leu	Ala	Ala	Arg	Asn	Gln	Glu	Pro	Asn	Glu	Arg	225	230	235	240
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<223> G767

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5	Met Met Lys Ser Gly Ala Asp Leu Gln Phe Pro Pro 1 5 10	
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	Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Leu Met Tyr Leu 15 20 25	
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	Cys Arg Lys Cys Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu 30 35 40	
	ctc gat ttg tac cga tat gat cct tgg gac ctt ccc gac atg gct ttg	255
	Leu Asp Leu Tyr Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu 45 50 55 60	
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	Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr 65 70 75	
20	cca aac ggt tca aga ccc aac cgt gca gct ggt act gga tat tgg aaa	351
	Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys 80 85 90	
	gct acc gga gct gat aaa cca ata ggt cgt cct aaa ccg gtt ggt att	399
	Ala Thr Gly Ala Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile 95 100 105	
25	aag aag gct cta gtg ttt tac tcg gga aaa cct cca aat gga gag aaa	447
	Lys Lys Ala Leu Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys 110 115 120	
	acc aat tgg att atg cac gaa tac cgg ctc gct gac gtt gac cgg tcg	495
	Thr Asn Trp Ile Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser 125 130 135 140	
30	ggt cgt aag aaa aac agt cta aga ttg gac gat tgg gta ttg tgt cgt	543
	Val Arg Lys Lys Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg 145 150 155	
35	ata tat aac aag aaa ggt gtc atc gag aag cga cga agc gat atc gag	591
	Ile Tyr Asn Lys Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu 160 165 170	
	gac ggg tta aag cct gtg act gac acg tgt cca ccg gaa tct gtg gcg	639
	Asp Gly Leu Lys Pro Val Thr Asp Thr Cys Pro Pro Glu Ser Val Ala 175 180 185	
40	aga ttg atc tcc ggc tcg gag caa gcg gtg tca ccg gaa ttc acg tgt	687
	Arg Leu Ile Ser Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys 190 195 200	
	agc aac ggt cgg ttg agt aat gcc ctt gat ttt ccg ttt aat tac gta	735
	Ser Asn Gly Arg Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val 205 210 215 220	
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	Asp Ala Ile Ala Asp Asn Glu Ile Val Ser Arg Leu Leu Gly Gly Asn 225 230 235	
50	cag atg tgg tcg acg acg ctt gat cca ctt gtg gtt agg cag gga act	831
	Gln Met Trp Ser Thr Thr Leu Asp Pro Leu Val Val Arg Gln Gly Thr 240 245 250	
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				20					25					30			
	Ala	Ser	Gln	Pro	Ile	Pro	Ala	Pro	Ile	Ile	Thr	Glu	Leu	Asp	Leu	Tyr	
15			35					40					45				
	Arg	Tyr	Asp	Pro	Trp	Asp	Leu	Pro	Asp	Met	Ala	Leu	Tyr	Gly	Glu	Lys	
		50					55					60					
20	Glu	Trp	Tyr	Phe	Phe	Ser	Pro	Arg	Asp	Arg	Lys	Tyr	Pro	Asn	Gly	Ser	
	65					70					75					80	
	Arg	Pro	Asn	Arg	Ala	Ala	Gly	Thr	Gly	Tyr	Trp	Lys	Ala	Thr	Gly	Ala	
					85					90					95		
25	Asp	Lys	Pro	Ile	Gly	Arg	Pro	Lys	Pro	Val	Gly	Ile	Lys	Lys	Ala	Leu	
				100					105					110			
	Val	Phe	Tyr	Ser	Gly	Lys	Pro	Pro	Asn	Gly	Glu	Lys	Thr	Asn	Trp	Ile	
30			115					120					125				
	Met	His	Glu	Tyr	Arg	Leu	Ala	Asp	Val	Asp	Arg	Ser	Val	Arg	Lys	Lys	
		130					135					140					
35	Asn	Ser	Leu	Arg	Leu	Asp	Asp	Trp	Val	Leu	Cys	Arg	Ile	Tyr	Asn	Lys	
	145					150					155					160	
	Lys	Gly	Val	Ile	Glu	Lys	Arg	Arg	Ser	Asp	Ile	Glu	Asp	Gly	Leu	Lys	
				165						170					175		
40	Pro	Val	Thr	Asp	Thr	Cys	Pro	Pro	Glu	Ser	Val	Ala	Arg	Leu	Ile	Ser	
				180					185					190			
	Gly	Ser	Glu	Gln	Ala	Val	Ser	Pro	Glu	Phe	Thr	Cys	Ser	Asn	Gly	Arg	
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	Leu	Ser	Asn	Ala	Leu	Asp	Phe	Pro	Phe	Asn	Tyr	Val	Asp	Ala	Ile	Ala	
		210					215					220					
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<223> G765

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 5 cgatctcttc aaaaagttat tgttttcttg aaggattttt cttgttcttg atcaagcata 180
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 Asp Gln Glu Val Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr
 15 20 25
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 Asp Glu Glu Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile
 30 35 40 45
 15 cga ttt acc gcg gca gcg att ggt caa gcc gac ctc aac aag aac gag 374
 Arg Phe Thr Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu
 50 55 60
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 Pro Trp Asp Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr
 20 65 70 75
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 Phe Phe Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn
 80 85 90
 25 cgt gcg acc gtg tct ggt tat tgg aag gcg acc ggg aag gac aag gag 518
 Arg Ala Thr Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu
 95 100 105
 atc ttt aga ggc aaa ggt tgt ctt gtt ggg atg aag aaa aca ctt gtt 566
 Ile Phe Arg Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val
 30 110 115 120 125
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 Phe Tyr Thr Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met
 130 135 140
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 His Glu Tyr Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys
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 Thr Ala Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala
 160 165 170
 40 cct agt act aca atc act act aca aaa caa ctc tca agg att gat tct 758
 Pro Ser Thr Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser
 175 180 185
 ctt gat aac att gat cat ctc tta gac ttc tca tct ctc cct cct ctc 806
 Leu Asp Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu
 190 195 200 205
 45 ata gat ccg ggt ttc ttg ggt caa ccc gcc caa gct tct ccg gtg ccc 854
 Ile Asp Pro Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro
 210 215 220
 gtc aac aac acg atc tca aac ctg tct cca cca tcc tac aac cgc acc 902
 Val Asn Asn Thr Ile Ser Asn Leu Ser Pro Pro Ser Tyr Asn Arg Thr
 50 225 230 235
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			240					245					250				
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	Leu	Gly	Pro														
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	ttgagttccg	atgtgaacac	aaccgcaacg	ccagagatat	cttcttatcc	aatgatgatg											1122
10	aatccggcaa	tgatggatgg	tagcaagtca	gcgtgtgatg	gtcttgatga	cttgatcttc											1182
	tgggaagatt	tatatactag	ctaaatttgg	gaaaaggtta	tttgtaatt	gtgattgaag											1242
	agtggcatat	tgattactcg	tctagtgttt	ttaatcgtgt	aattagttcg	tatataatat											1302
15	acatgtacat	aagatcatta	ggtttattag	gcattggact	ttagttcggg	gattgcttac											1362
	ctagtgttta	gcttgagaaa	aaaggctgtc	attggggtta	tgtttctttg	tgattaactt											1422
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5 Val Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu
20 25 30

Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile Arg Phe Thr
35 40 45

10 Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu Pro Trp Asp
50 55 60

Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe Cys
65 70 75 80

Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala Thr
85 90 95

20 Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg
100 105 110

Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr
115 120 125

25 Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met His Glu Tyr
130 135 140

Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys Thr Ala Arg
145 150 155 160

30 Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala Pro Ser Thr
165 170 175

35 Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser Leu Asp Asn
180 185 190

40 Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu Ile Asp Pro
195 200 205

Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro Val Asn Asn
210 215 220

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	Trp Thr Lys Glu Glu Asp Asp Lys Leu Ile Ser Tyr Ile Lys Ala His	
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15	ggg gaa ggt tgt tgg cgt tct ctt cct aga tcc gcc ggt ctt caa cgt	144
	Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg	
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	tgc gga aaa agc tgt cgt ctc cga tgg att aac tat ctc cga cct gat	192
	Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp	
	50 55 60	
20	ctc aag agg ggt aac ttc acc ctc gaa gaa gat gat ctc atc atc aaa	240
	Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys	
	65 70 75 80	
	cta cat agc ctt ctc ggt aac aag tgg tct ctt att gcg acg aga tta	288
	Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu	
	85 90 95	
25	cca gga aga aca gat aac gag att aag aat tac tgg aac aca cat gtt	336
	Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val	
	100 105 110	
	aag agg aag cta tta aga aaa ggg att gat ccg gcg act cat cga cct	384
	Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro	
	115 120 125	
30	atc aac gag acc aaa act tct caa gat tcg tct gat tct agt aaa aca	432
	Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr	
	130 135 140	
35	gag gac cct ctt gtc aag att ctc tct ttt ggt cct cag ctg gag aaa	480
	Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys	
	145 150 155 160	
	ata gca aat ttc ggg gac gag aga att caa aag aga gtt gag tac tca	528
	Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser	
	165 170 175	
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	180 185 190	
5	cca cca tgg caa gac aag ctc cat gat gag agg aac cta agg ttt ggg	624
	Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly	
	195 200 205	
	aga gtg aag tat agg tgc agt gcg tgc cgt ttt gga ttc ggg aac ggc	672
	Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly	
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	aag gag tgt agc tgt aat aat gtg aaa tgt caa aca gag gac agt agt	720
	Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser	
	225 230 235 240	
	agc agc agt tat tct tca acc gac att agt agt agc att ggt tat gac	768
	Ser Ser Ser Tyr Ser Ser Thr Asp Ile Ser Ser Ser Ile Gly Tyr Asp	
15	245 250 255	
	ttc ttg ggt cta aac aac act agg gtt ttg gat ttt agc act ttg gaa	816
	Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu	
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20 25 30

Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg
35 40 45

10 Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
50 55 60

15 Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys
65 70 75 80

Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu
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20 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val
100 105 110

Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro
115 120 125

25 Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr
130 135 140

30 Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys
145 150 155 160

Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser
165 170 175

35 Val Val Glu Glu Arg Cys Leu Asp Leu Asn Leu Glu Leu Arg Ile Ser
180 185 190

40 Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly
195 200 205

Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly
210 215 220

45 Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser
225 230 235 240

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 <223> G255

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	Lys Glu His Met Asn Lys Gly Ala Trp Thr Lys Glu Glu Asp Glu Arg	
	10 15 20	
	cta gtc tct tac atc aag tct cac ggt gaa ggt tgt tgg cga tct ctt	149
	Leu Val Ser Tyr Ile Lys Ser His Gly Glu Gly Cys Trp Arg Ser Leu	
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	cct aga gcc gct ggt ctc ctt cgc tgc ggt aaa agc tgc cgt ctt cgg	197
	Pro Arg Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser Cys Arg Leu Arg	
	45 50 55	
25	tgg att aac tat ctc cga cct gat ctc aaa aga gga aac ttt aca cat	245
	Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Asn Phe Thr His	
	60 65 70	
	gat gaa gat gaa ctt atc atc aag ctt cat agc ctc cta ggc aac aag	293
	Asp Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu Leu Gly Asn Lys	
	75 80 85	
30	tgg tct ttg att gcg gcg aga tta cct gga aga aca gat aac gag atc	341
	Trp Ser Leu Ile Ala Ala Arg Leu Pro Gly Arg Thr Asp Asn Glu Ile	
	90 95 100	
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	Ile	Asp	Pro	Ala	Thr	His	Arg	Gly	Ile	Asn	Glu	Ala	Lys	Ile	Ser	Asp	
					125					130					135		
	ttg	aag	aaa	aca	aag	gac	caa	att	gta	aaa	gat	gtt	tct	ttt	gtg	aca	485
	Leu	Lys	Lys	Thr	Lys	Asp	Gln	Ile	Val	Lys	Asp	Val	Ser	Phe	Val	Thr	
				140					145					150			
10	aag	ttt	gag	gaa	aca	gac	aag	tct	ggg	gac	cag	aag	caa	aat	aag	tat	533
	Lys	Phe	Glu	Glu	Thr	Asp	Lys	Ser	Gly	Asp	Gln	Lys	Gln	Asn	Lys	Tyr	
			155					160					165				
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15	Ile	Arg	Asn	Gly	Leu	Val	Cys	Lys	Glu	Glu	Arg	Val	Val	Val	Glu	Glu	
		170					175					180					
	aaa	ata	ggc	cca	gat	ttg	aat	ctt	gag	ctt	agg	atc	agt	cca	cca	tgg	629
	Lys	Ile	Gly	Pro	Asp	Leu	Asn	Leu	Glu	Leu	Arg	Ile	Ser	Pro	Pro	Trp	
		185				190					195					200	
20	caa	aac	cag	aga	gaa	ata	tct	act	tgc	act	gcg	tcc	cgt	ttt	tac	atg	677
	Gln	Asn	Gln	Arg	Glu	Ile	Ser	Thr	Cys	Thr	Ala	Ser	Arg	Phe	Tyr	Met	
				205						210					215		
	gaa	aac	gac	atg	gag	tgt	agt	agt	gaa	act	gtg	aaa	tgt	caa	aca	gag	725
	Glu	Asn	Asp	Met	Glu	Cys	Ser	Ser	Glu	Thr	Val	Lys	Cys	Gln	Thr	Glu	
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	aat	agt	agc	agc	att	agc	tat	tct	tct	att	gat	att	agt	agt	agt	aac	773
	Asn	Ser	Ser	Ser	Ile	Ser	Tyr	Ser	Ser	Ile	Asp	Ile	Ser	Ser	Ser	Asn	
			235				240						245				
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30	Val	Gly	Tyr	Asp	Phe	Leu	Gly	Leu	Lys	Thr	Arg	Ile	Leu	Asp	Phe	Arg	
		250				255						260					
	agc	ttg	gaa	atg	aaa	taa	atgaatagta	ttagattcct	aattttgtagg								869
	Ser	Leu	Glu	Met	Lys												
		265															
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Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu Thr Ser Arg Ser
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Asp Pro Thr Arg Leu Ala Leu Ser Thr Ser Ala Thr Leu Ala Asn Glu
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Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp Lys Ile Arg
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cag ctg ccg aag tgt gga cac gtg ttt cat cat cgt tgt tta gac cgt      392
Gln Leu Pro Lys Cys Gly His Val Phe His His Arg Cys Leu Asp Arg
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Trp Phe Arg Asp Glu Val Glu Ser Thr Asn
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Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu Thr
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65 70 75 80

Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp
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Leu Asp Arg Trp Ile Val Asp Cys Asn Lys Ile Thr Cys Pro Ile Cys
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gaa cac agt acc ctt aat cct aaa cag aag att gca ttg gcg aag cag      455
Glu His Ser Thr Leu Asn Pro Lys Gln Lys Ile Ala Leu Ala Lys Gln
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	Asp	Gly	Ser	Thr	Ala	Lys	Gly	Ala	Phe	Ser	Ile	Ser	Ser	Lys	Pro	His	
				250					255					260			
	ttc	ttc	aac	cct	ttt	act	aac	cca	tct	gca	gct	tgt	tga	atag	taatt		871
30	Phe	Phe	Asn	Pro	Phe	Thr	Asn	Pro	Ser	Ala	Ala	Cys					
			265				270										
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	<211> 274																
	<212> PRT																
	<213> Arabidopsis thaliana																
40	<400> 54																
45																	
50																	
55																	

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1 5 10 15

5 Pro Ser Pro Ile Ser Asn Asn Tyr Asn Ser Thr Ile Arg Gln Ser Ser
20 25 30

Val Tyr Lys Leu Glu Pro Ser Leu Thr Leu Cys Leu Ser Gly Asp Pro
35 40 45

10 Ser Val Thr Val Val Thr Gly Ala Asp Gln Leu Cys Arg Gln Thr Ser
50 55 60

15 Ser His Ser Gly Val Ser Ser Phe Ser Ser Gly Arg Val Val Lys Arg
65 70 75 80

Glu Arg Asp Gly Gly Glu Glu Ser Pro Glu Glu Glu Glu Met Thr Glu
85 90 95

20 Arg Val Ile Ser Asp Tyr His Glu Asp Glu Glu Gly Ile Ser Ala Arg
100 105 110

25 Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu Glu Glu Ser
115 120 125

Phe Lys Asp His Ser Thr Leu Asn Pro Lys Gln Lys Gln Val Leu Ala
130 135 140

30 Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn
145 150 155 160

35 Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe
165 170 175

Leu Lys Lys Cys Cys Glu Thr Leu Ala Asp Glu Asn Ile Arg Leu Gln
180 185 190

40 Lys Glu Ile Gln Glu Leu Lys Thr Leu Lys Leu Thr Gln Pro Phe Tyr
195 200 205

Met His Met Pro Ala Ser Thr Leu Thr Lys Cys Pro Ser Cys Glu Arg
210 215 220

45 Ile Gly Gly Gly Gly Gly Gly Asn Gly Gly Gly Gly Gly Ser Gly
225 230 235 240

Ala Thr Ala Val Ile Val Asp Gly Ser Thr Ala Lys Gly Ala Phe Ser
245 250 255

50 Ile Ser Ser Lys Pro His Phe Phe Asn Pro Phe Thr Asn Pro Ser Ala
260 265 270

55 Ala Cys

Claims

1. A transgenic plant having greater tolerance to an environmental stress than a wild-type plant of the same species, wherein the environmental stress is selected from the group consisting of drought, salt, heat and cold,
 wherein the transgenic plant comprises an expression vector or cassette, and wherein the expression vector or cassette comprises a polynucleotide having a sequence encoding a polypeptide having a conserved domain with at least 80% sequence identity to the conserved domain of amino acid residues 25 to 116 of SEQ ID NO:16, and wherein overexpression of the polypeptide in the transgenic plant increases the tolerance of the transgenic plant to the environmental stress as compared to the tolerance of the wild-type plant to drought, salt, heat or cold.
2. The transgenic plant of claim 1, wherein the polynucleotide has a sequence comprising SEQ ID NO:15.
3. The transgenic plant of any one of claims 1 or 2, wherein the expression vector or cassette further comprises a constitutive, inducible or tissue-specific promoter operably linked to the polynucleotide having said sequence.
4. The transgenic plant of any one of claims 1 to 3, wherein the transgenic plant has greater tolerance to salt than the wild-type plant.
5. The transgenic plant of any one of claims 1 to 4, wherein the plant is selected from the group consisting of soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits and vegetable brassicas.
6. The transgenic plant of any one of claims 1 to 5, wherein the transgenic plant is more tolerant than the wild type plant to a six-hour exposure to 200 mM NaCl.
7. A seed obtainable from the transgenic plant of any of claims 1 to 6, wherein the seed comprises the expression vector or cassette comprising the polynucleotide having said sequence.
8. Use of an expression vector or cassette to produce a transformed plant, wherein the expression vector or cassette encodes a polypeptide having a conserved domain with at least 80% sequence identity to the conserved domain of amino acid residues 25 to 116 of SEQ ID NO:16, and overexpression of the polypeptide in the transformed plant increases tolerance of the transformed plant to heat, drought, salt and/or cold as compared to the tolerance of the wild-type plant.
9. Use of a polynucleotide to produce a transformed plant, wherein the polynucleotide encodes a polypeptide having a conserved domain with at least 80% sequence identity to a conserved domain of amino acid residues 25 to 116 of SEQ ID NO:16, the polynucleotide is incorporated into an expression vector or cassette, the expression vector or cassette is introduced into a plant to produce the transformed plant, and the transformed plant has increased tolerance to heat, drought, salt and/or cold as compared to the tolerance of the wild type plant.
10. A method for producing a plant having increased tolerance to heat, drought, salt and/or cold, the method comprising the steps of:
 - (a) providing an expression vector or cassette comprising an isolated or recombinant polynucleotide encoding a polypeptide that comprises a conserved domain with at least 80% sequence identity to a conserved domain of amino acid residues 25 to 116 of SEQ ID NO:16; and
 - (b) introducing the expression vector or cassette into the plant to produce a transformed plant, wherein overexpression of the polypeptide in the transformed plant increases tolerance of the transformed plant to heat, drought, salt and/or cold as compared to the tolerance of a wild-type plant to drought, salt, heat and/or cold.
11. The method of claim 10, wherein the transformed plant is more tolerant than the wild type plant to a six-hour exposure to 200 mM NaCl.
12. The method according to claim 10 or 11, wherein the expression vector or cassette comprises a constitutive, inducible or tissue-specific promoter.

Patentansprüche

1. Transgene Pflanze mit höherer Toleranz gegenüber Umweltbelastungen als eine Wildtyppflanze derselben Spezies, worin die Umweltbelastung aus der aus Trockenheit, Salz, Hitze und Kälte bestehenden Gruppe ausgewählt ist, worin die transgene Pflanze einen Expressionsvektor oder eine Expressionskassette umfasst und worin der Expressionsvektor oder die Expressionskassette ein Polynucleotid mit einer Sequenz umfasst, die für ein Polypeptid kodiert, das eine konservierte Domäne mit zumindest 80 % Sequenzidentität mit der konservierten Domäne der Aminosäurereste 25 bis 116 der Seq.-ID Nr. 16 aufweist, und worin eine Überexpression des Polypeptids in der transgenen Pflanze die Toleranz der transgenen Pflanze gegenüber Umweltbelastungen im Vergleich zur Toleranz der Wildtyppflanze gegenüber Trockenheit, Salz, Hitze oder Kälte steigert.
2. Transgene Pflanze nach Anspruch 1, worin das Polynucleotid eine Sequenz aufweist, die Seq.-ID Nr. 15 umfasst.
3. Transgene Pflanze nach einem der Ansprüche 1 bis 2, worin der Expressionsvektor oder die Expressionskassette weiteres einen konstitutiven, induzierbaren oder gewebespezifischen Promotor umfasst, der operabel an das Polynucleotid mit der genannten Sequenz gebunden ist.
4. Transgene Pflanze nach einem der Ansprüche 1 bis 3, worin die transgene Pflanze höhere Toleranz gegenüber Salz aufweist als die Wildtyppflanze.
5. Transgene Pflanze nach einem der Ansprüche 1 bis 4, worin die Pflanze aus der aus Sojabohne, Weizen, Mais, Kartoffel, Baumwolle, Reis, Raps, Sonnenblume, Luzerne, Zuckerrohr, Rasen, Banane, Brombeere, Heidelbeere, Erdbeere, Himbeere, Cantaloupe-Melone, Karotte, Karfiol, Kaffee, Gurke, Melanzani, Weintrauben, Honigmelone, Gartensalat, Mango, Zuckermelone, Zwiebel, Papaya, Erbsen, Paprika, Ananas, Spinat, Kürbis, Süßmais, Tabak, Tomate, Wassermelone, Rosengewächs-Obstbäume und Gemüsekohlarten bestehenden Gruppe ausgewählt ist.
6. Transgene Pflanze nach einem der Ansprüche 1 bis 5, worin die transgene Pflanze gegenüber einer sechsstündigen Aussetzung gegenüber 200 mM NaCl toleranter ist als die Wildtyppflanze.
7. Samen, der von einer transgenen Pflanze nach einem der Ansprüche 1 bis 6 erhältlich ist, worin der Samen den Expressionsvektor oder die Expressionskassette umfasst, der/die das Polynucleotid mit der genannten Sequenz umfasst.
8. Verwendung eines Expressionsvektors oder einer Expressionskassette, um eine transformierte Pflanze herzustellen, worin der Expressionsvektor oder die Expressionskassette für ein Polypeptid kodiert, das eine konservierte Domäne mit zumindest 80 % Sequenzidentität mit der konservierten Domäne der Aminosäurereste 25 bis 116 der Seq.-ID Nr. 16 aufweist, und eine Überexpression des Polypeptids in der transformierten Pflanze die Toleranz der transformierten Pflanze gegenüber Hitze, Trockenheit, Salz und/oder Kälte im Vergleich zur Toleranz der Wildtyppflanze steigert.
9. Verwendung eines Polynucleotids zur Herstellung einer transformierten Pflanze, worin das Polynucleotid für ein Polypeptid kodiert, das eine konservierte Domäne mit zumindest 80 % Sequenzidentität mit der konservierten Domäne der Aminosäurereste 25 bis 116 der Seq.-ID Nr. 16 aufweist, das Polynucleotid in einen Expressionsvektor oder eine Expressionskassette inkorporiert ist, der Expressionsvektor oder die Expressionskassette in eine Pflanze eingeführt wird, um die transformierte Pflanze herzustellen, und die transformierte Pflanze im Vergleich zur Toleranz der Wildtyppflanze gesteigerte Toleranz gegenüber Hitze, Trockenheit, Salz und/oder Kälte aufweist.
10. Verfahren zur Herstellung einer Pflanze mit gesteigerter Toleranz gegenüber Hitze, Trockenheit, Salz und/oder Kälte, wobei das Verfahren folgenden Schritte umfasst:
 - (a) das Bereitstellen eines Expressionsvektors oder einer Expressionskassette, der/die ein isoliertes oder rekombinantes Polynucleotid umfasst, das für ein Polypeptid kodiert, welches eine konservierte Domäne mit zumindest 80 % Sequenzidentität mit der konservierten Domäne der Aminosäurereste 25 bis 116 von Seq.-ID Nr. 16 aufweist; und
 - (b) das Einführen des Expressionsvektors oder der Expressionskassette in die Pflanze, um eine transformierte Pflanze herzustellen, worin

eine Überexpression des Polypeptids in der transformierten Pflanze die Toleranz der transformierten Pflanze gegenüber Trockenheit, Salz, Hitze und/oder Kälte im Vergleich zur Toleranz der Wildtyppflanze Trockenheit, Salz, Hitze und/oder Kälte steigert.

11. Verfahren nach Anspruch 10, worin die transformierte Pflanze gegenüber einer sechsstündigen Aussetzung gegenüber 200 mM NaCl toleranter ist als die Wildtyppflanze.
12. Verfahren nach Anspruch 10 oder 11, worin der Expressionsvektor oder die Expressionskassette einen konstitutiven, induzierbaren oder gewebespezifischen Promoter umfasst.

Revendications

1. Plante transgénique ayant une plus grande tolérance vis-à-vis d'un stress environnemental qu'une plante du type sauvage de la même espèce, où le stress environnemental est sélectionné dans le groupe consistant en sécheresse, sel, chaleur et froid, où la plante transgénique comprend un vecteur d'expression ou cassette et où le vecteur d'expression ou cassette comprend un polynucléotide ayant une séquence codant pour un polypeptide ayant un domaine conservé avec au moins 80% d'identité de séquence avec le domaine conservé de résidus d'acides aminés 25 à 116 de SEQ ID NO:16; et où la surexpression du polypeptide dans la plante transgénique augmente la tolérance de la plante transgénique au stress environnemental en comparaison avec la tolérance de la plante du type sauvage vis-à-vis de la sécheresse, du sel, de la chaleur ou du froid.
2. Plante transgénique de la revendication 1, où le polynucléotide a une séquence comprenant SEQ ID NO:15.
3. Plante transgénique de l'une quelconque des revendications 1 ou 2, où le vecteur d'expression ou cassette comprend de plus un promoteur constitutif inductible spécifique du tissu opérativement enchaîné au polynucléotide ayant ladite séquence.
4. Plante transgénique de l'une quelconque des revendications 1 à 3, où la plante transgénique a une plus grande tolérance vis-à-vis d'un sel que la plante du type sauvage.
5. Plante transgénique de l'une quelconque des revendications 1 à 4, où la plante est sélectionnée dans le groupe consistant en soja, blé, maïs, pomme de terre, coton, riz, colza, tournesol, luzerne, canne à sucre, gazon, banane, mûre, myrtille, fraise, framboise, cantaloup, carotte, choux-fleur, café, concombre, aubergine, raisin, honeydew, laitue, mangue, melon, oignon, papaye, pois, poivre, ananas, épinard, patisson, maïs doux, tabac, tomate, melon d'eau, fruits rosacés, brassicas végétaux.
6. Plante transgénique de l'une quelconque des revendications 1 à 5, où la plante transgénique est plus tolérante que la plante du type sauvage vis-à-vis d'une exposition de six heures à NaCl 200 mM.
7. Graine obtenue à partir de la plante transgénique de l'une quelconque des revendications 1 à 6, où la graine comprend le vecteur d'expression ou cassette comprenant le polynucléotide ayant ladite séquence.
8. Utilisation d'un vecteur d'expression ou d'une cassette pour produire une plante transformée où le vecteur d'expression ou cassette code pour un polypeptide ayant un domaine conservé avec au moins 80% d'identité de séquence avec le domaine conservé des résidus d'acides aminés 25 à 116 de SEQ ID NO:16 et la surexpression du polypeptide dans la plante transformée augmente la tolérance de la plante transformée à la chaleur, à la sécheresse, au sel et/ou au froid en comparaison avec la tolérance du type sauvage.
9. Utilisation d'un polynucléotide pour produire une plante transformée où le polynucléotide code pour un polypeptide ayant un domaine conservé avec au moins 80% d'identité de séquence avec un domaine conservé de résidus d'acides aminés 25 à 116 de SEQ ID NO:16, le polynucléotide est incorporé dans un vecteur d'expression ou cassette, le vecteur d'expression ou cassette est introduit dans une plante pour produire la plante transformée et la plante transformée a une tolérance accrue à la chaleur, à la sécheresse, au sel et/ou au froid en comparaison avec la tolérance de la plante du type sauvage.
10. Méthode de production d'une plante ayant une tolérance accrue à la chaleur, à la sécheresse, au sel et/ou au froid,

la méthode comprenant les étapes de :

(a) prévoir un vecteur d'expression ou cassette comprenant un polynucléotide isolé ou recombinant codant pour un polypeptide qui comprend un domaine conservé avec au moins 80% d'identité de séquence avec un domaine conservé des résidus d'acides aminés 25 à 116 de SEQ ID NO:16 ; et

(b) introduire le vecteur d'expression ou cassette dans la plante pour produire une plante transformée, où

la surexpression du polypeptide dans la plante transformée augmente la tolérance de la plante transformée à la chaleur, la sécheresse, le sel et/ou le froid en comparaison avec la tolérance d'une plante du type sauvage à la sécheresse, le sel, la chaleur et/ou le froid.

11. Méthode de la revendication 10, où la plante transformée est plus tolérante que la plante du type sauvage à une exposition de six heures à NaCl 200 mM.

12. Méthode selon la revendication 10 ou 11, où le vecteur d'expression ou cassette comprend un promoteur constitutif inductible spécifique du tissu.

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G22	cDNA	
2	G22	protein	89-157
3	G188	cDNA	
4	G188	protein	175-222
5	G225	cDNA	
6	G225	protein	39-76
7	G226	cDNA	
8	G226	protein	28-78
9	G256	cDNA	
10	G256	protein	13-115
11	G419	cDNA	
12	G419	protein	392-452
13	G464	cDNA	
14	G464	protein	7-15,70-80,125-158,183-219
15	G482	cDNA	
16	G482	protein	25-116
17	G502	cDNA	
18	G502	protein	10-155
19	G526	cDNA	
20	G526	protein	21-149
21	G545	cDNA	
22	G545	protein	82-102, 136-154
23	G561	cDNA	
24	G561	protein	248-308
25	G664	cDNA	
26	G664	protein	13-116
27	G682	cDNA	
28	G682	protein	22-53
29	G911	cDNA	
30	G911	protein	86-129
31	G964	cDNA	
32	G964	protein	126-186
33	G394	cDNA	
34	G394	protein	121-182
35	G489	cDNA	
36	G489	protein	57-156

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
37	G463	homolog of G464	cDNA	
38	G463	homolog of G464	protein	14-23, 77-88, 130-146, 194-227
39	G767	homolog of G502	cDNA	
40	G767	homolog of G502	protein	8-158
41	G765	homolog of G526	cDNA	
42	G765	homolog of G526	protein	23-167
43	G197	homolog of G664	cDNA	
44	G197	homolog of G664	protein	14-119
45	G255	homolog of G664	cDNA	
46	G255	homolog of G664	protein	14-115
47	G1113	homolog of G911	cDNA	
48	G1113	homolog of G911	protein	85-128
49	G398	homolog of G964	cDNA	
50	G398	homolog of G964	protein	128-191
51	G395	homolog of G394	cDNA	
52	G395	homolog of G394	protein	72-135
53	G393	homolog of G394	cDNA	
54	G393	homolog of G394	protein	106-169

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G22	790359	1.00E-45	Nicotiana tabacum
1	G22	3342210	6.60E-45	Lycopersicon esculentum
1	G22	6654776	1.60E-44	Medicago truncatula
1	G22	8809570	5.80E-44	Nicotiana sylvestris
1	G22	7627061	2.40E-39	Gossypium arboreum
1	G22	7324479	9.50E-36	Lycopersicon pennellii
1	G22	8980312	4.30E-31	Catharanthus roseus
1	G22	7528275	1.20E-30	Mesembryanthemum crystallinum
1	G22	6478844	4.60E-28	Matricaria chamomilla
1	G22	6847348	5.90E-26	Glycine max
3	G188	7779802	5.20E-36	Lotus japonicus
3	G188	7284340	2.10E-34	Glycine max
3	G188	9361307	1.20E-27	Triticum aestivum
3	G188	7340336	1.10E-22	Oryza sativa
3	G188	6529152	3.60E-22	Lycopersicon esculentum
3	G188	8748477	7.70E-21	Medicago truncatula
3	G188	5456433	7.10E-14	Zea mays
3	G188	9302479	1.60E-12	Sorghum bicolor
3	G188	6696287	4.10E-12	Pinus taeda
3	G188	562242	9.00E-12	Brassica rapa
5	G225	4396287	4.40E-16	Glycine max
5	G225	309571	0.00029	Zea mays
5	G225	3857004	0.001	Populus tremula x Populus tremuloides
5	G225	9410205	0.019	Triticum aestivum
5	G225	9426190	0.025	Triticum turgidum subsp. durum
5	G225	8382118	0.046	Gossypium arboreum
5	G225	6782756	0.27	Oryza sativa
5	G225	7721017	0.4	Lotus japonicus
5	G225	6020136	0.47	Pinus taeda
5	G225	2921331	0.48	Gossypium hirsutum
7	G226	4396287	5.10E-15	Glycine max
7	G226	9410205	1.50E-05	Triticum aestivum
7	G226	3857004	0.11	Populus tremula x Populus tremuloides
7	G226	2428139	0.35	Oryza sativa
9	G256	1430847	1.30E-72	Lycopersicon esculentum
9	G256	9252441	1.20E-65	Solanum tuberosum
9	G256	8380712	2.20E-58	Gossypium arboreum
9	G256	8172976	1.60E-54	Medicago truncatula
9	G256	9205295	1.30E-44	Glycine max
9	G256	20562	6.40E-40	Petunia x hybrida
9	G256	4886263	4.40E-37	Antirrhinum majus
9	G256	6552360	5.00E-36	Nicotiana tabacum
9	G256	2312003	1.20E-35	Oryza sativa
9	G256	5268628	5.20E-35	Zea mays
11	G419	7239156	2.60E-59	Malus x domestica
11	G419	5278451	9.00E-58	Lycopersicon esculentum
11	G419	9205496	1.30E-55	Glycine max
11	G419	7628137	9.30E-51	Gossypium arboreum
11	G419	6069643	9.50E-51	Oryza sativa
11	G419	7562931	9.80E-45	Medicago truncatula
11	G419	7322293	2.30E-37	Lycopersicon hirsutum
11	G419	8404716	1.10E-29	Hordeum vulgare
11	G419	7217755	1.40E-29	Sorghum bicolor

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G419	9428023	4.60E-28	<i>Triticum aestivum</i>
13	G464	6527230	3.60E-31	<i>Lycopersicon esculentum</i>
13	G464	9305572	1.10E-22	<i>Sorghum bicolor</i>
13	G464	6604917	6.70E-22	<i>Medicago truncatula</i>
13	G464	5058123	2.30E-21	<i>Glycine max</i>
13	G464	3760881	1.20E-19	<i>Oryza sativa</i>
13	G464	5044476	1.20E-17	<i>Gossypium hirsutum</i>
13	G464	9412603	6.40E-15	<i>Triticum aestivum</i>
13	G464	7777277	3.20E-13	<i>Lotus japonicus</i>
13	G464	9410371	1.70E-11	<i>Hordeum vulgare</i>
13	G464	7624108	2.10E-10	<i>Gossypium arboreum</i>
15	G482	7691987	5.50E-50	<i>Glycine max</i>
15	G482	7781090	1.30E-48	<i>Lotus japonicus</i>
15	G482	7409616	1.10E-47	<i>Lycopersicon esculentum</i>
15	G482	9416562	4.40E-46	<i>Triticum aestivum</i>
15	G482	22379	2.30E-44	<i>Zea mays</i>
15	G482	7501372	7.70E-44	<i>Gossypium arboreum</i>
15	G482	7765436	8.40E-42	<i>Medicago truncatula</i>
15	G482	5044464	1.20E-40	<i>Gossypium hirsutum</i>
15	G482	9441376	9.20E-40	<i>Chlamydomonas reinhardtii</i>
15	G482	8071558	3.50E-39	<i>Solanum tuberosum</i>
17	G502	6730941	1.60E-91	<i>Oryza sativa</i>
17	G502	7765679	1.60E-82	<i>Medicago truncatula</i>
17	G502	7502501	7.30E-80	<i>Gossypium arboreum</i>
17	G502	5510359	8.30E-77	<i>Glycine max</i>
17	G502	5601137	8.70E-76	<i>Lycopersicon esculentum</i>
17	G502	9302206	1.40E-73	<i>Sorghum bicolor</i>
17	G502	4089948	3.40E-50	<i>Brassica napus</i>
17	G502	8329134	7.90E-49	<i>Mesembryanthemum crystallinum</i>
17	G502	7723564	8.60E-49	<i>Lotus japonicus</i>
17	G502	4218534	1.80E-48	<i>Triticum sp.</i>
19	G526	5049217	3.40E-61	<i>Gossypium hirsutum</i>
19	G526	6066594	1.50E-55	<i>Petunia x hybrida</i>
19	G526	4384535	1.50E-54	<i>Lycopersicon esculentum</i>
19	G526	6454868	6.60E-54	<i>Glycine max</i>
19	G526	4977542	4.70E-52	<i>Oryza sativa</i>
19	G526	5343151	7.00E-51	<i>Zea mays</i>
19	G526	9361647	5.10E-50	<i>Triticum aestivum</i>
19	G526	6799764	4.30E-48	<i>Medicago truncatula</i>
19	G526	8708684	1.80E-47	<i>Hordeum vulgare</i>
19	G526	4218536	3.60E-47	<i>Triticum sp.</i>
21	G545	4666359	8.30E-55	<i>Datisca glomerata</i>
21	G545	7228328	3.70E-52	<i>Medicago sativa</i>
21	G545	1763062	1.30E-51	<i>Glycine max</i>
21	G545	7206360	3.10E-44	<i>Medicago truncatula</i>
21	G545	7626808	9.60E-40	<i>Gossypium arboreum</i>
21	G545	439492	3.90E-39	<i>Petunia x hybrida</i>
21	G545	4382658	1.70E-38	<i>Lycopersicon esculentum</i>
21	G545	8486215	8.70E-38	<i>Euphorbia esula</i>
21	G545	7322653	6.80E-37	<i>Lycopersicon hirsutum</i>
21	G545	7785845	1.10E-33	<i>Lotus japonicus</i>
23	G561	2995461	5.60E-86	<i>Sinapis alba</i>
23	G561	633153	6.50E-83	<i>Brassica napus</i>

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
23	G561	1033058	5.90E-65	Raphanus sativus
23	G561	2815304	2.10E-35	Spinacia oleracea
23	G561	1498300	1.60E-34	Petroselinum crispum
23	G561	169958	8.10E-32	Glycine max
23	G561	5381310	2.20E-30	Catharanthus roseus
23	G561	1155053	9.70E-28	Phaseolus vulgaris
23	G561	728627	1.90E-27	Nicotiana tabacum
23	G561	7565950	1.40E-21	Medicago truncatula
25	G664	1167483	4.90E-81	Lycopersicon esculentum
25	G664	7765706	6.30E-69	Medicago truncatula
25	G664	19052	9.30E-68	Hordeum vulgare
25	G664	7626566	4.00E-67	Gossypium arboreum
25	G664	5050757	2.60E-66	Gossypium hirsutum
25	G664	6850206	6.90E-66	Oryza sativa
25	G664	6667606	2.20E-63	Glycine max
25	G664	517492	9.30E-62	Zea mays
25	G664	9302672	1.50E-59	Sorghum bicolor
25	G664	5860031	9.20E-58	Pinus taeda
27	G682	309571	4.40E-08	Zea mays
27	G682	4396287	1.10E-05	Glycine max
27	G682	3857004	0.00051	Populus tremula x Populus tremuloides
27	G682	9410205	0.00085	Triticum aestivum
27	G682	8382118	0.0079	Gossypium arboreum
27	G682	2428139	0.017	Oryza sativa
27	G682	7339148	0.13	Lycopersicon esculentum
27	G682	9302672	0.32	Sorghum bicolor
27	G682	5048991	0.39	Gossypium hirsutum
27	G682	6555777	0.46	Pinus taeda
29	G911	4090113	6.10E-51	Brassica napus
29	G911	5893315	7.70E-25	Lycopersicon esculentum
29	G911	5048452	3.10E-23	Gossypium hirsutum
29	G911	9440241	1.90E-21	Glycine max
29	G911	6917169	1.80E-11	Lycopersicon pennellii
29	G911	9297970	3.20E-11	Sorghum bicolor
29	G911	7137594	4.90E-11	Zea mays
29	G911	9278447	4.60E-10	Lotus japonicus
29	G911	7560271	7.20E-10	Medicago truncatula
29	G911	5043346	4.50E-09	Sorghum halepense
31	G964	7624806	3.30E-72	Gossypium arboreum
31	G964	1234899	9.10E-66	Glycine max
31	G964	1149534	1.50E-61	Pimpinella brachycarpa
31	G964	8919872	3.40E-51	Capsella rubella
31	G964	992597	6.70E-51	Lycopersicon esculentum
31	G964	1235564	1.50E-38	Oryza sativa
31	G964	6605613	3.00E-32	Medicago truncatula
31	G964	1032371	4.50E-28	Helianthus annuus
31	G964	3868846	2.80E-25	Ceratopteris richardii
31	G964	8088109	6.40E-22	Sorghum bicolor
33	G394	8670502	7.90E-59	Glycine max
33	G394	3171738	2.00E-54	Craterostigma plantagineum
33	G394	1032371	1.10E-50	Helianthus annuus
33	G394	7624806	4.30E-47	Gossypium arboreum
33	G394	1160483	2.10E-46	Pimpinella brachycarpa

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
33	G394	3868846	4.20E-45	Ceratopteris richardii
33	G394	992597	1.10E-44	Lycopersicon esculentum
33	G394	7558511	1.50E-44	Medicago truncatula
33	G394	8099247	6.20E-43	Oryza sativa
33	G394	8919872	1.20E-40	Capsella rubella
35	G489	6534956	4.40E-62	Lycopersicon esculentum
35	G489	9055852	2.60E-60	Medicago truncatula
35	G489	8382393	6.20E-51	Gossypium arboreum
35	G489	8789169	2.10E-50	Citrus x paradisi
35	G489	9252957	1.50E-47	Soianum tuberosum
35	G489	6918056	4.70E-47	Lycopersicon pennellii
35	G489	7590809	1.00E-46	Glycine max
35	G489	5257255	8.60E-43	Oryza sativa
35	G489	4152190	3.20E-41	Zea mays
35	G489	6069260	2.10E-39	Ceratodon purpureus
37	G463	6527230	4.90E-36	Lycopersicon esculentum
37	G463	9305572	5.50E-36	Sorghum bicolor
37	G463	3760881	1.20E-31	Oryza sativa
37	G463	6604917	1.30E-23	Medicago truncatula
37	G463	5058123	2.50E-21	Glycine max
37	G463	5044476	1.10E-19	Gossypium hirsutum
37	G463	9412603	1.70E-17	Triticum aestivum
37	G463	9419394	6.00E-17	Hordeum vulgare
37	G463	7624108	6.20E-17	Gossypium arboreum
37	G463	8547152	3.20E-16	Nicotiana tabacum
39	G767	5510359	2.80E-76	Glycine max
39	G767	7643155	4.20E-74	Medicago truncatula
39	G767	6977319	1.10E-72	Lycopersicon esculentum
39	G767	6730939	4.20E-68	Oryza sativa
39	G767	7502501	2.00E-67	Gossypium arboreum
39	G767	9302206	3.10E-65	Sorghum bicolor
39	G767	4218534	4.30E-51	Triticum sp.
39	G767	6732157	4.30E-51	Triticum monococcum
39	G767	9412602	6.90E-47	Triticum aestivum
39	G767	8329134	1.30E-46	Mesembryanthemum crystallinum
41	G765	4384535	3.10E-56	Lycopersicon esculentum
41	G765	6454868	8.50E-56	Glycine max
41	G765	1279639	4.30E-53	Petunia x hybrida
41	G765	4977542	2.00E-51	Oryza sativa
41	G765	4218536	2.00E-50	Triticum sp.
41	G765	6732159	2.00E-50	Triticum monococcum
41	G765	5049217	6.90E-50	Gossypium hirsutum
41	G765	9361647	4.50E-49	Triticum aestivum
41	G765	9296257	2.90E-48	Sorghum bicolor
41	G765	8708684	4.30E-46	Hordeum vulgare
43	G197	1167483	2.70E-76	Lycopersicon esculentum
43	G197	7626566	2.40E-73	Gossypium arboreum
43	G197	7765706	1.50E-63	Medicago truncatula
43	G197	19052	8.90E-63	Hordeum vulgare
43	G197	5050757	1.60E-62	Gossypium hirsutum
43	G197	6850206	1.10E-61	Oryza sativa
43	G197	6667606	1.70E-61	Glycine max
43	G197	517492	7.60E-59	Zea mays

Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
43	G197	5860031	3.90E-57	<i>Pinus taeda</i>
43	G197	9302672	3.80E-55	<i>Sorghum bicolor</i>
45	G255	1167483	6.40E-75	<i>Lycopersicon esculentum</i>
45	G255	7626566	6.40E-71	<i>Gossypium arboreum</i>
45	G255	19050	2.80E-65	<i>Hordeum vulgare</i>
45	G255	5050757	3.70E-63	<i>Gossypium hirsutum</i>
45	G255	7590249	4.10E-62	<i>Glycine max</i>
45	G255	7765706	4.40E-62	<i>Medicago truncatula</i>
45	G255	6850206	1.10E-61	<i>Oryza sativa</i>
45	G255	517492	3.50E-59	<i>Zea mays</i>
45	G255	9302672	1.60E-56	<i>Sorghum bicolor</i>
45	G255	7721017	2.60E-55	<i>Lotus japonicus</i>
47	G1113	4090113	2.30E-36	<i>Brassica napus</i>
47	G1113	5048452	6.80E-12	<i>Gossypium hirsutum</i>
47	G1113	5893315	9.50E-11	<i>Lycopersicon esculentum</i>
47	G1113	9440241	7.70E-09	<i>Glycine max</i>
49	G398	7624806	2.80E-67	<i>Gossypium arboreum</i>
49	G398	1234899	6.90E-64	<i>Glycine max</i>
49	G398	1149534	6.20E-63	<i>Pimpinella brachycarpa</i>
49	G398	8919872	2.60E-47	<i>Capsella rubella</i>
49	G398	992597	1.10E-39	<i>Lycopersicon esculentum</i>
49	G398	1235564	7.70E-39	<i>Oryza sativa</i>
49	G398	6605613	1.70E-33	<i>Medicago truncatula</i>
49	G398	8088109	3.60E-33	<i>Sorghum bicolor</i>
49	G398	3868846	1.60E-32	<i>Ceratopteris richardii</i>
49	G398	3171738	1.00E-27	<i>Craterostigma plantagineum</i>
51	G395	992597	5.30E-51	<i>Lycopersicon esculentum</i>
51	G395	7624806	2.00E-50	<i>Gossypium arboreum</i>
51	G395	1234899	1.50E-49	<i>Glycine max</i>
51	G395	1165131	1.90E-48	<i>Pimpinella brachycarpa</i>
51	G395	3868846	3.40E-47	<i>Ceratopteris richardii</i>
51	G395	7415619	1.30E-41	<i>Physcomitrella patens</i>
51	G395	8919872	7.40E-41	<i>Capsella rubella</i>
51	G395	1235564	2.70E-38	<i>Oryza sativa</i>
51	G395	8088109	2.30E-33	<i>Sorghum bicolor</i>
51	G395	1032371	3.30E-31	<i>Helianthus annuus</i>
53	G393	8670502	3.60E-55	<i>Glycine max</i>
53	G393	9199975	7.60E-46	<i>Medicago truncatula</i>
53	G393	3868846	9.60E-37	<i>Ceratopteris richardii</i>
53	G393	8919872	2.50E-35	<i>Capsella rubella</i>
53	G393	7624806	1.30E-34	<i>Gossypium arboreum</i>
53	G393	7415619	1.00E-33	<i>Physcomitrella patens</i>
53	G393	5897000	5.50E-33	<i>Lycopersicon esculentum</i>
53	G393	1235564	4.00E-32	<i>Oryza sativa</i>
53	G393	1165131	6.40E-32	<i>Pimpinella brachycarpa</i>
53	G393	3171738	1.50E-31	<i>Craterostigma plantagineum</i>

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